SEARCH FOR THE POTENTIAL APPLICATION OF FRUIT PEEL WASTES AS NATURAL BIOPESTICIDES FOR THE CONTROL AND MANAGEMENT OF *PAPILIO DEMOLEUS* WITH REFERENCE TO POPULATION SUPPRESSION

Research Protocol

For Registration for Ph.D Programme

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CERTIFICATE

This is to certify that this Research Proposal entitled "**Search for the potential application of fruits peel waste as natural biopesticides for the control and management of *Papilio demoleus* with reference to population suppression"** by **Arti Khati,** submitted in fulfillment of the requirement for registration in Ph.D. program in **Department of Zoology** under Faculty of Sciences of JECRC University, Jaipur, during the academic year 2020, is a bonafide record of work carried out under my guidance and supervision.

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INTRODUCTION:

India, which is an agronomic country as more than 80% population is dependent on agriculture and agriculture productivity decides the economy of India. However, agriculture productivity is diminished by more than 45% before or after harvesting due to attacks of a variety of pests like insects, nematodes, viruses and bacteria induced diseases and competition by weeds (David, 2008).

To reduce these disadvantages and protect the agriculture from the attack of insects, pests, plant pathogens, weeds, etc. pesticides are used frequently. Pesticides are substances or mixture of substances which are used to prevent, suppress, resist, kill, sterilize, or reduce pests and have become an important part of human life used for agricultural build up. To protect the agriculture and to increase the productivity large amount of fertilizers, insecticides, pesticides, biocides, herbicides and other soil additives are being used indiscriminately. The indiscriminate and unplanned use of chemical pesticides may result in acute and chronic poisoning, deterioration of the environment and development of resistant in the pest strains (Ali *et al*. 2008). So, it becomes our need to find out some alternatives so as to minimize and overcome these harmful effects of chemical pesticides by utilizing some safe alternative. Poor farmers in India employ a range of old historic methods such as ash; sand dry pepper and plant extracts**.**

So the alternative (which can reduce negative impacts on health of the people and nature) can be the use of biological pesticides as these are obtained from some product of nature such as fauna, flora and bacteria, or specific minerals (Nelson & Washington, 2004). Easy to use, unharmed so can be used for the control of different crop/fruit pests.

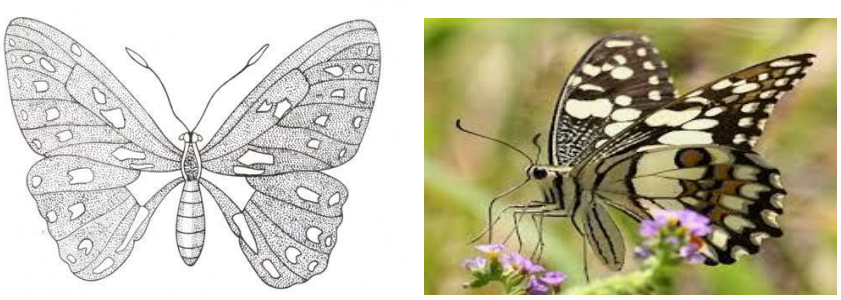
Plants are kept plants, produce a wide variety of antibacterial, antifungal and pesticidal compounds in their constitutive due to which they are able to protect themselves from the attack of many invaders (Cammue *et al.,* 1992). Beside these plants also produces alkaloids, flavonoids, tannins, phenols, terpenoids, glycosides etc. as secondary metabolites or phytochemicals for their defense mechanism and to

protect them from the attack of insect-pests (Ahmad, 2007). These phytocompounds have been reported to show repellent (Schmutterer, 1995), larvicidal (Kabaru and Gichia, 2001), antifeedant (Raja *et al.,* 2005), ovicidal and oviposition deterrent (Pavunraj *et al.,* 2006), activity in the insects/pests. Some phytocompounds may affect juvenile hormone and ecdysone actions or may disrupt insect growth by antagonizing juvenile hormone action (Williams *et al.,* 1986). Thus, plant pesticides or biopesticides in comparison to chemical pesticides are not so costly, environmentally safe and extremely effective in controlling pest infestation (Matthews, 1999; Nathan & Kalaivani, 2005).

Papilio demoleus **(the test organism)**

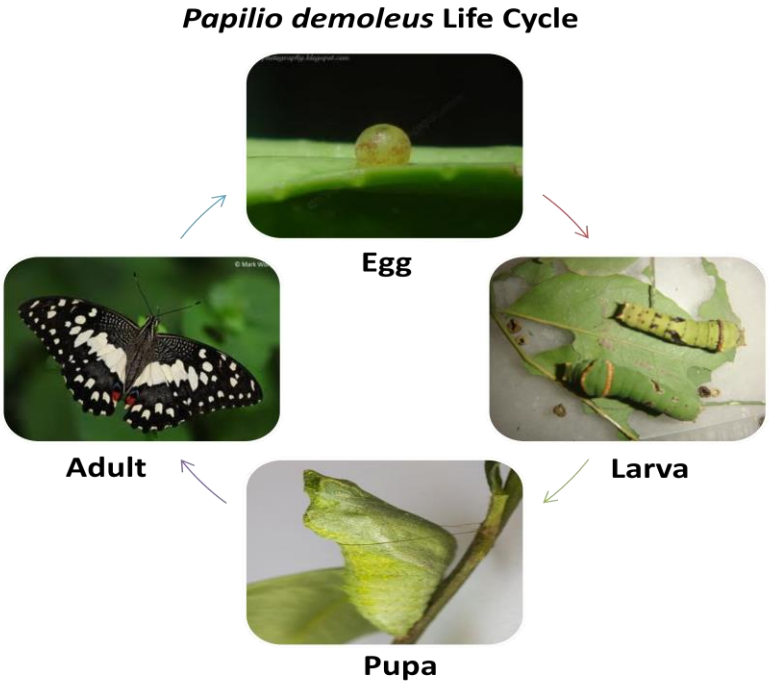
Systematic position:
Kigdom-Animalia
Phylum-Arthropoda
Sub-phylum- Mandibulata
Class-Insecta
Sub-class-Pterygota
Order-Lepidoptera
Family-Papilionidae
Genus-*Papilio*
Species-*demoleus*

Papilio demoleus (Lemon butterfly or Citrus caterpillar) is a widespread insect pest which is found in large number in countries like India, Pakistan, Bangladesh, Iran, China, and North-Australia. It (especially larval phase) is a serious pest of citrus plants as it is a vigorous foliage feeder and causes potential damage to the plants and thus to citrus industry. Insect pest *Papilio demoleus* also destroys the quality and palatability of lemon. So the protection of citrus trees against these insect pests has been a major problem for the development of agriculture.



Papilio demoleus

Life cycle of *Papilio demoleus* involves different phases i.e. egg, larva, pupa and adult. Its life span is completed in 15 days (summer) and about 47 days (winter). Life span of an adult male is about 135 days while that of the female is about 145 days. The duration of their life stages differs with the locality and may have eight generations per year. The other stages of the life cycle may have the following time durations viz. - egg: 3.1– 6.1 days, larva: 12.9 – 22.7 days, pupa: 8.0 – 22.4 days, and adult: 4.0 – 6.0 days (Badawi & ali, 1981).



Host plants

The major host plants of *Papilio demoleus* includes *Citrus aurantium* (bitter orange), *Citrus aurantifola* (Key lime) (Kunte and Krushnamegh, 2000), *Glycosmis pentaphylla* (Jamaica

mandarin orange), *Ruta graveolens* (Rue), *Aegle marmelos* (Bael fruit), *Murraya koenigii* (curry-leaf), and *Chloroxylon swietenia* (East Indian satinwood) (Wynter-Blyth, 1957).

Nature of damage and control:-

Larva being the rapacious feeder of tender leaves, feed leaves inward from their boundary. Younger plants cannot withstand loss of foliage and dies. Where infestation levels are low, larva are also regulated by hand picking (Cherian, 1942). But for high and severe infestations, the chemical pesticides like carbaryl, phosalone, acephate, etc. are generally used (Radke and Kandalkar, 1986; Batra 1990; Solunke and Deshpande 1991).

Keeping the above facts in mind (i.e. to search for safer and cheaper alternatives to chemical insecticides/pesticides), the present study, **"Search for the potential application of waste fruit peels (jackfruit, pomegranate) as natural biopesticides for the control and management of *Papilio demoleus* with reference to population suppression"** will be evaluated.

For this study the peels of the following fruits will be taken, which are selected on the basis of their potential phytochemicals and availability.

- **Jackfruit (*Artocarpus heterophyllus*) (Family: Moraceae)**

Jack fruit is village cultivated and is one of the popular fruit of India. Both ripe and unripe fruit pulp are consumed in different ways. However, the peels are discarded after the separation of the fruit pod. As such there is no commercial use of its peel. Alkaloids, flavonoids, carbohydrate, protein and triterpenoids as effective phytocompounds and secondary metabolites like hexadecanoic acid squalene calophyllolide thialisophyine have been detected in ethanolic and methanolic extracts of jack fruit peels (Sundarraj and

Ranganathan, 2017). The pulp flake and seed extract in comparison to peel extract contains less amount of total phenolic and total flavonoid compound. Peel extract also contain glucosidase hindrance activity (about 11.8 folds of that of a carbase) (Zhang *et.al*, 2017).

The jackfruit contains many medicinal properties within it, due to which it is very famous. In China, jackfruit pulp and seeds are deemed as a chilling and nourishing stimulant, which neutralizes the effect of alcohol. Jackfruit has many medicinal properties like antimalarial, anti arthritic, hypoglycemic, anti diarrheal, anti inflammatory, anti platelets, antiviral, wound healing, anticancer, and anti helminthic (Tejpal and Amrita, 2016). Due to the presence of flavonoids, it causes the possible mortality of insects (Catherine *et al*., 2009). Jackfruit peel contains tannin which is also flavored with bitter polyphenols, which makes the jackfruit peels tasteless for many insect pests and it provide protection against its predators (War *et al.*, 2012). So tannin has an adaptative contribution in the plant defense to different distress situation. Therefore, these may be used as potential insecticide (McGee, 2004).

- **Pomegranate (*Punica granatum*) (Family: Lythraceae)**

Pomegranate is an important fruit crop of Lythraceae family. It is generally consumed as a juice extract from seeds of the pomegranate. After juice extraction a nutritive byproduct, that is, pomegranate peel is left. It also hold medicinal property such as Antibacterial, Anticancer, anti-oxidants, wound healing property, antivirus, anti proliferative activities (Navarro *et al* .1996; Chidambara *et al*. 2004; Adhami *et al*. 2006; Rajan *et. al*.2011). It is highly used for effective diet composition, primarily for heart and prostate health.

The peel of the pomegranate possesses a variety of phytochemical compounds like flavonoid, polyphenol, ellagitannins compounds, minerals (calcium, phosphorus, nitrogen, potassium, magnesium, sodium), and polysaccharide (Kumar &.Neeraj, 2018). According to previous studies phytochemicals have deleterious effect against insect in various ways like growth

retardation, suppression of calling, toxicity and reduction of fertility. It also contains flavonoid compounds which are familiar to modulate the feeding and oviposition behavior of insects and thus, they can be used as dominant insecticide (Golawaska and Lukasik, 2012).

As we know, that some solvents might possess harmful side-effects on human being organs. Thus the major objective of my present study is the insecticidal activities of jackfruit and pomegranate peels to mimic the nature conditions for insecticides administration. The antimicrobial potency of both, abundantly available fruits peel wastes (jackfruit, pomegranate) and flavonoid, tannin are the most powerful tools to control insects.

Rajasthan is a member of the citrus fruits highly producing states in India but infestation of this pest (*Papilio demoleus*) results in severe economic losses. To protect the plants from attack of the pest and indiscriminate use of chemical pesticide is done which is harmful for the human health and environment. So as to minimize these harmful effects search for a biological pesticide becomes necessary. Beside this the fruit peels are generally considered as wastes although they possess higher contents of many phytocompounds and secondary metabolites which can be utilized for the control of pest population as the presence of these compounds protects the plants from predation in a natural way.

Thus, the present study is undertaken to evaluate the potential use of waste fruit peels (jackfruit and pomegranate) as natural biopesticides for the control and management of *Papilio demoleus* with reference to population suppression**.**

PART LITERATURE REVIEW

Related literature and studies done by different researchers are as follows-

- Fawzi, Nasir, and Layla, (2007) studied effect of *Bacillus thuringiensis* var. kuristaki Berliner as biological control against, *Papilio demoleus* under the laboratory and reported a direct relationship between used bacterial concentration and mortality rate. They also observed 75% mortality and some relating to development deformities at 0.25 mg/ml concentration, 100% mortality at 0.5 mg/ml and 100% mortality at 1.0 mg/ml.

Due to the use of bacterial application, resulted in increase of duration of the developmental period and decrease in egg production (Fawzi *et al.*, 2007).

- Vattikonda *et al*., (2014) studied potential activity of *Ziziphus jujuba* plant extract for the management of *Papilio demoleus* larvae. He evaluated betulinic acid isolated from the bark of the plant for its effect on the feeding activity in relation to food utilization at different concentrations of 50, 100, 150, 200 ppm in 4th instar larvae of *P. demoleus* and reported that the antifeeding activity of Betulinic acid was most vigorous against all development stages of *P. demoleus*. A significant (94.04%) mortality was found at 200ppm concentration. Thus, suggesting that Betulinic acid can replace the synthetic pesticides since it is safer to the environment and biodegradable in nature.

- Vattikonda *et al*. (2014) also evaluated Forskolin (a plant extract, which was isolated from the root of *Coleus forskohlii*) for its antifeedant activity of citrus leaves by *P. demoleus* larvae and found the antifeeding activity of Forskolin proved to be the most potent against all development stages of *P. demoleus*. Vattikonda *et al.* (2017) also observed the impact of this extract on the fertility and reproductive potency of *P. demoleus* and suggested that forskolin can be used as a good antifertility product for the control of pest.

- Ben Hamouda *et al*., (2014) reported the extract of the pomegranate against developmental stages (larva and adult) of the red flour beetle *Tribolium castaneum* and observed highest larval mortalities in methanol extract and a poor anti-feeding activity in methanol extract. Thus, suggesting the use of plant based pesticide in providing protection against coleopteran pest infections in stored grain.

- Radwan, Sekina & Emam (2016) reported that pomegranate peels and leave crude can be used as natural insecticides after the results obtained from the study. They reported the presence of steroids, tannins, phenolic compounds in the extracts which show a great impact on insecticidal activities.

- Kumar *et al.,* (2016) studied the efficacy of fruit peel wastes against chikungunya vector, *Aedes aegypti* and observed the hexane extracts of peels to be more potent than petroleum ether extracts with regard to the citrus species. The hexane extract of *C.*

sinensis peels was found to be very effective larvicidal factor for *Aedes aegypti* whereas petroleum ether peels extract of *C. limon* was the most effective larvicidal agent against *A. aegypti*, and that of *C. limetta* peel wastes showed least activity. A complete mortality was obtained in all treated group whereas no mortality was shown by any control or untreated groups within 24 hours.

- Gupta *et al*., (2017) studied on utilization of fruit peel and seeds aqueous extract of the wild pomegranate for the management of rose aphids and reported a highly significant percent mortalities of Aphids at all the doses of the extract used.

- Vattikonda *et al*., (2018) studied the effect of Andrographolide (a plant extract, which was isolated from the stem and leaves of the *Andrographis paniculata*) on ovarian development of *Papilio demoleus* and found the disturbance of ovarian structure and inhibition of oocyte growth that caused fast surcease of oviposition.

- Liwayway, (2019) studied insecticidal property of ethanol extract of Jackfruit (*Artocarpus heterophyllus*) peel against Rice Weevils (*Sitophilus oryzae*) and found that different concentration of the extract affects the insect mortality. Many phytochemical compounds and secondary metabolites present in the extract of the fruit peel alter some biochemical functions of organisms resulting in its mortality.

The part literature review shows that many plants show the insecticidal activity on many insect pests and fruit peel extracts have been used for the control of different stored pests or mosquitoes. The effect of fruit peel on the developmental stages of *Papilio demoleus* and their role in its mortality is lacking, hence the aim of the present study is that can jackfruit and pomegranate fruit peel wastes extracts be used as a natural insecticide for the control or suppression of population of *Papilio demoleus.*

To the best of our knowledge as such no work related to toxicological study of fruit peel waste on developmental stages of *Papilio demoleus* has been done in Rajasthan. So the purpose of the study is an effort to study the pesticidal potential of different extracts of the

selected fruit peel wastes and further identifying and suggesting different methods of suppression of population of *Papilio demoleus.*

STATEMENT OF RESEARCH PROBLEM

Control and Management of *Papilio demoleus* With Reference to Population Suppression Using Fruit Peel Wastes

Management includes preparation, organization and commanding a structure to solve and manage all kinds of pest problem.

Population Suppression – to decrease the fertility rate .

OBJECTIVES OF THE STUDY -

1. To collect, screen and identify test fruit peel wastes and culturing of pest.
2. To evaluate the toxicological effect of the extracts of jack fruit peel wastes on developmental stages (i.e. egg, larvae, pupa and adult) of *Papilio demoleus.*
3. To evaluate the toxicological effect of the extracts of pomegranate peel wastes on developmental stages (i.e. egg, larvae, pupa and adult) of *Papilio demoleus.*
4. To search and evaluate different methods of management and suppression of population of *Papilio demoleus.*

Methodology -

To carry out my research work the following methodology would be undertaken:

1. **Collection and maintenance of culture of *Papilio demoleus***:

For the purpose of the research work the culture of the test organism (i.e. *Papilio demoleus*) will be maintained throughout the study. For which initially different growing stages of pest like that egg, larva, pupae and adults will be gathered from the fresh lemon plantation and the nurseries in sterile jars with no history of pesticide use and brought to Zoology laboratory, JECRC University. All the experimental work will be carried out under standard laboratory conditions.

The colony of *Papilio demoleus* will be maintained in an insectory under controlled conditions. Initially an adult paired *Papilio demoleus* will be fed on a piece of cotton dipped in 10% sugar solution inside mosquito net as the source of food for feeding and then eggs will be collected daily to have the different developmental stages for the study (Wail *et al.*, 2009). Then fresh lemon plant from nursery with few twigs up to two feet will be kept in each mosquito cage for oviposition.

2. Collection of fruit peel wastes:

The fruits of jackfruit and pomegranate will be collected from the surrounding area/markets of Jaipur (Rajasthan, India) and from the kitchen garden in sterilized polythene bags. The peels will be removed from both selected fruits and will be completely washed first with tap water and then with rectified alcohol. We will also observe that there is no infection and no any kind of disease in fruit peel is present. We will remove the infected fruit peel, and the fresh healthy selected peels will be maintained for dryness at room temperature of 27 **°C**. The dried fruit peels will be ground to coarse powder in a mechanical grinder using the small blender. The grounded coarse powder will be sieved so as to get fine powder for the purpose of extraction.

3. Preparation of peels extract:

The following extract will be made from the fruit peels:

A) **Crude extraction**- The obtained fine powder of the selected fruit peels will be macerated and soaked in different solvents (viz. petroleum ether (B.P. 60-80°C), ethanol and distilled water) as follows the ratio of 1:4w/v for three days with constant agitation. After three days the mixture will be filtered through cheese cloth and whatman paper no.1 and will then be allowed to evaporate in a rotary vacuum evaporator. GCMS will also be done to find out the presence of pesticide residues and bioactive compounds in fruit peels extract. The crude extract thus obtained will be stored till further experimentation.

B) **Soxhlet extraction**- The powdered form of fruit peels will then be processed for extraction by successively soxhleting them with petroleum ether (B.P. 60-80°C), ethanol and distilled water. The crude extracts obtained will be evaporated to dryness or concentrated under low temperature and reduced pressure by standard procedure.

The collected concentrated extract will be further diluted to the desired concentration to perform the pesticidal assay.The stock extract will be stored in the refrigerator and maintained at 5±1°C temperature till further use.

4. Toxicity and mortality study (Pesticidal study):

For the pesticidal studies, four pairs of sterilized petridishes will be used. Then fruit peels extract will be diluted with distilled water so as to have different concentration gradient. After this the fresh citrus leaf will be dipped in the fruit peels extract from both sides uniformly and placed on moist filter paper in a Petridish. Single larva of *Papilio demoleus* will be introduced into the same petridish. Controls of *Papilio demoleus* will also be maintained simultaneously. 10 such Petridishes will be taken for each experiment and then will be observed at 24, 48 and 72 h for toxicological effects. Thus different developmental stages (i.e. egg laid, larvae, pupa and adult) of pest will be treated with gradient concentration of the fruit peel extracts in triplicate petriplates. Progressive consumption of leaf area by the larva will be observed and measured using leaf area meter after 24 hours. And will be compared with the control. The percentage of antifeedant activity will be calculated using the formula of Ben *et al*., (2000).

$$\text{Antifeedant activity} = \frac{\text{Leaf area consumed in control} - \text{treated leaf}}{\text{Leaf area consumed in control} + \text{treated leaf}} * 100$$

The experiments will be performed thrice to get the mean value and standard deviation. The dead larva will be observed after 24 h for the larval mortality. Serial quantities of the fruit peels extract will be used to determine LC25 and LC50 for each extract.

The toxicological activity of the fruit peel extracts individually, as well as in combination will be studied by Thin Layer Film technique (contact toxicity) (Ascher & Mirian, 1981).

5. **Population management**

In the pesticidal activity effect on mortality, abnormality in larvae, pupae as well as adult and molting deformities, wing shape, pigmentation, body weight and changes in time duration of developmental stages of the pest will be studied.

The data obtained will be analyzed statistically for the management and suppression of the population of the pest. **Statistical analysis** will be performed using SPSS statistical software. The statistical significance of differences between individual means will be determined by student "t" test for paired observations.

EXPECTED OUTCOME OF THE STUDY

- The data obtained from the study will provide combined and comparative information of pesticidal effect of extracts of fruit peel wastes on the life cycle of the pest and suggesting some effective measures for minimizing and to protect citrus plants against insect pest (*Papilio demoleus*) by the use of potential biopesticides.
- The study will help in creating awareness to farmers and general public for the use of biological pesticides instead of chemical pesticides.
- This study will also provide guidelines for the management and suppression of population of the pest by controlling various developmental stages of the pest, *Papilio demoleus*

OUTLAY

Collection of literature and standardization of technique

⇓

Maintaining pure culture of pest and collection of test fruit peel wastes

⇓

Preparation of extracts in different solvents (petroleum ether, alcohol, aqueous)

⇓

Biopesticidal study of extracts of test plant material & Population management

⇩

Compilation, Analysis and Interpretation of data

⇩

Thesis writing and submission of Report

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